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(54) Title: DISCRIMINATION BETWEEN ANTIBODIES AGAINST HTLV-I, HTLV-II OR RELATED RETROVIRUSES, NEW PEPTIDES, DETECTION OF ANTIBODIES AND IMMUNOASSAY KITS

(57) Abstract

A method of discriminating between specific antibodies in samples of sera or other body fluids from humans or other primates containing antibodies arising from infection with HTLV-I, containing antibodies arising from infection with HTLV-II or containing antibodies arising from infection with related retroviruses, is described. In said method, the sample to be analyzed is subjected to at least four immunoassays, each using a different diagnostic antigen selected from four defined groups of peptides. Additionally, an immunoassay kit adapted for said method of discrimination, new peptides and a method of detecting antibodies with said peptides, are described.

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DISCRIMINATION BETWEEN ANTIBODIES AGAINST HTLV-I, HTLV-II OR RELATED RETROVIRUSES, NEW PEPTIDES, DETECTION OF ANTIBODIES AND IMMUNOASSAY KITS

The present invention relates to a method of discriminating between specific antibodies in samples of sera or other body fluids from humans or other primates containing antibodies arising from infection with HTLV-I, HTLV-II or related retroviruses. Additionally, it relates to an immunoassay kit adapted for said method of discrimination, and new peptides and a method of detecting antibodies with said peptides.

BACKGROUND

Up to now the following techniques for differentiating infection with the two viruses have been used: Virus isolation with typing, serological techniques (based on antibody competition or neutralization), or nucleic acid techniques (nucleic acid amplification or hybridization). Most of these techniques are laborious and require special competence.

Human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II) are widespread human retroviruses (a short review is given in ref. 6) (1, 2, 3, 20). HTLV-II has for several years been considered to be rare, but has recently proved to be a rather common infection among intravenous drug abusers primarily in the United States of America. The viruses cross-react serologically. It is therefore impossible to discriminate between an infection with one virus from an infection with the other with current antibody tests. It may prove clinically important to differentiate between infections with the two viruses. HTLV-I is associated with a type of leukemia (Adult T cell Leukemia; ATL) while HTLV-II has been observed in a few cases of hairy cell leukemia. There is a need for simple tests to differentiate between the two infections.

Even if the amino acid sequences of HTLV-I and HTLV--II proteins are similar there are several regions where

they are markedly different. Our idea is to use synthetic peptides from such regions as antigens in antibody tests. We have found peptides with sequences which e.g. are suitable for solid phase immunoassays and which give a type-specific antibody reactivity. We have found techniques where we use them to discern infection with HTLV-I from infection with HTLV-II.

DESCRIPTION OF THE INVENTION

One aspect of the invention is directed to a method of discriminating between specific antibodies in samples of sera or other body fluids from humans or other primates containing antibodies arising from infection with HTLV-I, containing antibodies arising from infection with HTLV-II or containing antibodies arising from infection with related retroviruses, whereby the sample to be analyzed is subjected to at least four immunoassays, each using a different diagnostic antigen selected from the following groups a) to d):

a) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I gag comprising antigenic structures;

b) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II gag comprising antigenic structures;

c) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I env comprising antigenic structures;

d) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II env comprising antigenic structures;

with the proviso that at least one peptide from each of the groups a) to d) is selected and, further, that at least one pair of peptides corresponding to at least

partially overlapping sequences of HTLV-I and HTLV-II is selected from each of the groupages a) plus b), and c) plus d),

and that the analyzed, different binding strengths of the antibodies of the sample in said at least four immuno-assays are used to discriminate between antibodies arising from infection with one specific retrovirus and antibodies arising from infection with other specific retroviruses.

In an embodiment of this aspect of the invention the diagnostic antigens are selected in the above manner from the peptides:

a) HTLV-I gag 130-197 PVMHPHGAPPNHRPWQMKDLQAIKQEVSQAAPGSPQFMQTIRLAVQQFDPTAKDLQDLLQYLCSSLVA
b) HTLV-II gag 137-214 PILHPPGAPSAHRPWQMKDLQAIKQEVSSSALGSPQFMQTLRLAVQQFDPTAKDLQDLLQYLCSSLVV

a) HTLV-I gag 298-349 LRSLAYSNANKECQKLLQARGHTNSPLGDMLRACQTWTPKDKTKVLVVQPKK
b) HTLV-II gag 305-356 LRSLAYSNANKECQKILQARGHTNSPLGEMLRTCQAWTPKDKTKVLVVQPRR

a) HTLV-I gag 4-20 IFSRSASPIPRPPRGLA
b) HTLV-II gag 4-20 IHGLSPTPIPKAPRGLS

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

a) HTLV-I gag 265-285 SILQGLEEPYHAFVERLNIAL

a) HTLV-I gag 302-320 LAYSNANKECQKLLQARGH

a) HTLV-I gag 323-341 SPLGDMLRACQTWTPKDKT

a) HTLV-I gag 337-355 PKDKTKVLVVQPKKPPPNQ
b) HTLV-II gag 343-361 PKDKTKVLVVQPRRPPPTQ

a) HTLV-I gag 378-399 PCPLCQDPTHWKRDCPRLKPT

a) HTLV-I gag 392-411 DCPRLKPTIPEPEPEEDALL
b) HTLV-II gag 398-416 DCPQLKPPQEEGEPLLLDL

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

c) HTLV-I env 290-312 HNSLILPPFSLSPVPTLGSRSRR

c) HTLV-I env 360-378 AIVKNHKNLLKIAQYAAQN

c) HTLV-I env 376-392 AQNRRGLDLLFWEQGGL

c) HTLV-I env 380-398 RGLDLLFWEQGGLCKALQE

c) HTLV-I env 465-488 RQLRHLPSRVRYPHYSLILPESSL
d) HTLV-II env 463-486 IQALPQRLQNRHNQYSLINPETML

In a preferred embodiment at least the following peptides are selected:

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

In a further preferred embodiment the sample to be analyzed is subjected to at least eight immunoassays and the analyzed pattern of binding strengths is processed with a computer program.

Optionally, at least one of the selected peptides is attached to an inert soluble or insoluble carrier.

Another aspect of the invention is directed to a peptide, which corresponds to a sequence of HTLV-I, HTLV-II or a related retrovirus each comprising antigenic structures and which comprises a sequence of at least 17 amino acid residues selected from the following sequences:

HTLV-I gag 130-197 PVMHPHGAPPNHRPWQMKDLQAIKQEVSQAAPGSPQFMQTIRLAVQQFDPTAKDLQDLLQYLCSSLVA

HTLV-II gag 137-214 PILHPPGAPSAHRPWQMKDLQAIKQEVSSSALGSPQFMQTLRLAVQQFDPTAKDLQDLLQYLCSSLVV

HTLV-I gag 298-349 LRSLAYSNANKECQKLLQARGHTNSPLGDMLRACQTWTPKDKTKVLVVQPKK
HTLV-II gag 305-356 LRSLAYSNANKECQKILQARGHTNSPLGEMLRTCQAWTPKDKTKVLVVQPRR

HTLV-I gag 4-20 IFSRSASPIPRPPRGLA
HTLV-II gag 4-20 IHGLSPTPIPKAPRGLS

HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

HTLV-I gag 265-285 SILQGLEEPYHAFVERLNIAL

HTLV-I gag 302-320 LAYSNANKECQKLLQARGH

HTLV-I gag 323-341 SPLGDMLRACQTWTPKDKT

HTLV-I gag 337-355 PKDKTKVLVVQPKKPPPNQ
HTLV-II gag 343-361 PKDKTKVLVVQPRRPPPTQ

HTLV-I gag 378-399 PCPLCQDPTHWKRDCPRLKPT

HTLV-I gag 392-411 DCPRLKPTIPEPEPEEDALL
HTLV-II gag 398-416 DCPQLKPPQEEGEPLLLDL

HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

HTLV-I env 290-312 HNSLILPPFSLSPVPTLGSRSRR

HTLV-I env 360-378 AIVKNHKNLLKIAQYAAQN

HTLV-I env 376-392 AQNRRGLDLLFWEQGGL

HTLV-I env 380-398 RGLDLLFWEQGGLCKALQE

HTLV-I env 465-488 RQLRHLPSRVRYPHYSLILPESSL

HTLV-II env 463-486 IQALPQRLQNRHNQYSLINPETML

Yet another aspect of the invention is directed to a method of detecting antibodies arising from infection with HTLV-I, HTLV-II or a related retrovirus in a sample of serum or other body fluid from a human or an other primate, whereby said sample is subjected to an immunoassay using as a diagnostic antigen at least one peptide of the invention.

Still another aspect of the invention is directed to an immunoassay kit for the discrimination between samples of sera or other body fluids from humans or other primates containing antibodies arising from infection with HTLV-I, containing antibodies arising from infection with HTLV-II or containing antibodies arising from infection with related retroviruses, which kit comprises at least four peptides selected from the following groups a) to d):

a) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I gag comprising antigenic structures;

b) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II gag comprising antigenic structures;

c) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I env comprising antigenic structures;

d) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II env comprising antigenic structures;

with the proviso that it comprises at least one peptide from each of the groups a) to d) and, further, that it comprises at least one pair of peptides corresponding to at least partially overlapping sequences of HTLV-I and

HTLV-II from each of the groupages a) plus b), and c) plus d).

In an embodiment of this aspect of the invention the immunoassay kit comprises at least four peptides selected in the above manner from the peptides:

a) HTLV-I gag 130-197 PVMHPHGAPPNHRPWQMKDLQAIKQEVSQAAPGSPQFMQTIRLAVQQFDPTAKDLQDLLQYLCSSLVA
b) HTLV-II gag 137-214 PILHPPGAPSAHRPWQMKDLQAIKQEVSSSALGSPQFMQTLRLAVQQFDPTAKDLQDLLQYLCSSLVV

a) HTLV-I gag 298-349 LRSLAYSNANKECQKLLQARGHTNSPLGDMLRACQTWTPKDKTKVLVVQPKK
b) HTLV-II gag 305-356 LRSLAYSNANKECQKILQARGHTNSPLGEMLRTCQAWTPKDKTKVLVVQPRR

a) HTLV-I gag 4-20 IFSRSASPIPRPPRGLA
b) HTLV-II gag 4-20 IHGLSPTPIPKAPRGLS

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

a) HTLV-I gag 265-285 SILQGLEEPYHAFVERLNIAL

a) HTLV-I gag 302-320 LAYSNANKECQKLLQARGH

a) HTLV-I gag 323-341 SPLGDMLRACQTWTPKDKT

a) HTLV-I gag 337-355 PKDKTKVLVVQPKKPPPNQ
b) HTLV-II gag 343-361 PKDKTKVLVVQPRRPPPTQ

a) HTLV-I gag 378-399 PCPLCQDPTHWKRDCPRLKPT

a) HTLV-I gag 392-411 DCPRLKPTIPEPEPEEDALL
b) HTLV-II gag 398-416 DCPQLKPPQEEGEPLLLDL

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

c) HTLV-I env 290-312 HNSLILPPFSLSPVPTLGSRSRR

c) HTLV-I env 360-378 AIVKNHKNLLKIAQYAAQN

c) HTLV-I env 376-392 AQNRRGLDLLFWEQGGL

c) HTLV-I env 380-398 RGLDLLFWEQGGLCKALQE

c) HTLV-I env 465-488 RQLRHLPSRVRYPHYSLILPESSL
d) HTLV-II env 463-486 IQALPQRLQNRHNQYSLINPETML

In a preferred embodiment of this aspect of the invention the immunoassay kit comprises at least the following peptides:

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

Short description of the drawings

Figure 1. Distribution of antibody reactivity with the peptide pair 1GB/2GB. Data from 15 HTLV-I and 10 HTLV-II positive sera from USA. Filled circles=HTLV-II positive sera.

Figure 2. Distribution of antibody reactivity with the peptide pair 1EA/2EA. Symbols and sera as in Figure 1.

Figure 3. Classification of serological reactivity with the help of the computer program HTLVPARS. HTLV-I and HTLV-II points have been computed with the same sera as in Figures 1 and 2, and are shown with the same symbols.

One-letter code for amino acids.

In the specification and claims the following conventional one-letter code is used:

A Alanine
C Cysteine
D Aspartic acid

E Glutamic acid
F Phenylalanine
G Glycine
H Histidine
I Isoleucine
K Lysine
L Leucine
M Methionine
N Asparagine
P Proline
Q Glutamine
R Arginine
S Serine
T Threonine
V Valine
W Tryptophan
Y Tyrosine

MATERIAL

Synthetic peptides

The following peptides were synthesized. The letters to the left in the following symbolize the peptides employed.

1GA HTLV-I gag 4-20 IFSRSASPIPRPPRGLA
2GA HTLV-II gag 4-20 IHGLSPTPIPKAPRGLS

1GB HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
2GB HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

1GC HTLV-I gag 265-285 SILQGLEEPYHAFVERLNIAL

1GD HTLV-I gag 302-320 LAYSNANKECQKLLQARGH

1GE HTLV-I gag 323-341 SPLGDMLRACQTWTPKDKT

2GF HTLV-I gag 337-355 PKDKTKVLVVQPKKPPPNQ

1GG	HTLV-II	gag	343-361	PKDKTKVLVVQPRRPPPTQ
1GH	HTLV-I	gag	378-399	PCPLCQDPTHWKRDCPRLKPT
1GI	HTLV-I	gag	392-411	DCPRLKPTIPEPEPEEDALL
2GI	HTLV-II	gag	398-416	DCPQLKPPQEEGEPLLLDL
1EA	HTLV-I	env	190-213	LLPHSNLDHILEPSIPWKSKLLTL
2EA	HTLV-II	env	186-209	LVHDSDLEHVLTPSTSWTTKILKF
1EB	HTLV-I	env	290-312	HNSLILPPFSLSPVPTLGSRSRR
1EC	HTLV-I	env	360-378	AIVKNHKNLLKIAQYAAQN
1ED	HTLV-I	env	376-392	AQNRRGLDLLFWEQGGL
1EE	HTLV-I	env	380-398	RGLDLLFWEQGGLCKALQE
1EF	HTLV-I	env	465-488	RQLRHLPSRVRYPHYSLILPESSL
2EF	HTLV-II	env	463-486	IQALPQRLQNRHNQYSLINPETML

The peptides were synthesized with a solid-phase technique according to the FMOC technology on an Applied Biosystems 430A machine. They were purified to 99.5% purity on a C18 column in an HPLC chromatograph, and were characterized by analytical HPLC, amino acid sequencing and amino acid analysis.

Sera

We used sera from 4 HTLV-I seropositive patients with adult T cell leukemia (a gift from dr Yorio Hinuma, Japan), one HTLV-I seropositive patient with tropical spactic paraparesis (TSP; an ethiopian immigrant to Sweden), five STLV-I antibody positive cynomolgus monkeys (found by us during testing of a large number of monkey sera, cf (5)), 15 HTLV-I seropositive intravenous drug abusers from the USA (sera typed with competitions RIPA (17, 25); a gift from dr Marjorie Robert-Guroff, National Cancer Institute, USA). We

used 38 sera from Swedish blood donors as negative controls.

Immunoenzymatic antibody determination

We utilized an enzymatic antibody detection technique (Enzyme immunoassay; EIA) where the synthetic HTLV peptides dissolved at a concentration of 20 µg/ml were allowed to adsorb from a volume of 100 µl to an activated plastic surface, and thereafter allowed to react with antibodies in a patient serum, followed by enzyme(peroxidase) labelled indicator antibodies. The technique corresponds to the one we have described earlier (4, 15). As a measure of the serological reactivity (the IgG activity) directed against the respective synthetic peptide we used the difference in absorbance at 450 nm between a peptide-coated and a not-peptide-coated microplate well which had been incubated with the same serum at a dilution of 1/50.

RESULTS

Table 1a. In a series of 35 sera with known or probable specificity the analyses yielded the results shown below. The figures are the absorbance difference between peptide-coated and not-peptide-coated well in EIA. Only results from peptides which gave a clear and specific reactivity (absorbance difference of >=0.3, and an absence of reactivity with the negative controls) are shown:

1GB	2GB	2GF	1EA	2EA	1EB	1EC	1ED	1EE	1EF	2EF	Our result	Known /Probable type
Sera from four patients with adult T-cell leukemia.												
1.4	0.9	0.0	1.1	0.3	0.0	0.4	0.3	0.5	0.0	0.0	1	(1)
1.1	0.2	0.0	0.7	0.2	0.0	0.2	0.0	0.0	0.2	0.0	1	(1)
0.8	0.7	0.1	0.4	0.1	0.0	0.3	0.0	0.3	0.5	0.1	1	(1)
0.9	1.1	0.1	0.3	0.2	0.0	0.6	0.2	0.3	0.0	0.0	1	(1)
Serum from one patient with tropical spastic paraparesis.												
0.6	0.1	0.2	1.5	0.2	0.5	0.0	0.0	0.4	0.0	0.0	1	(1)
Sera from five STLV-I positive cynomolgus monkeys.												
1.7	1.3	0.6	0.4	0.1	0.0	0.0	0.0	0.3	0.0	0.0	1	(1)
1.6	0.4	0.0	0.3	0.1	0.0	0.2	0.4	0.0	0.0	0.0	1	(1)
1.6	0.3	0.1	1.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	1	(1)
1.5	0.0	0.0	1.1	0.3	0.0	0.0	0.2	0.4	0.0	0.0	1	(1)
1.5	0.5	0.0	0.3	0.1	0.0	0.6	0.0	0.0	0.0	0.0	1	(1)

Table 1b. Sera from 25 intravenous drug abusers, and 6 negative control sera, all from the USA (25). These sera were analyzed blindly. The results from one serum constitute one row.

1GB	2GB	2GF	1EA	2EA	1EB	1EC	1ED	1EE	1EF	2EF	Our result	Known/ Probable type
0	0	0.1	0.2	0	0	0.1	0.2	0.1	0.2	0.1	1	1
0.8	0.8	0	1.0	0.5	1.1	0.4	1.0	1.4	0.3	0	1	1
0	0	0.1	0.1	0.6	0.1	0	0.2	0.4	0.1	0.4	2	2
0	0	0	.1	0	0	0	0	0	0	0	0	0
0.8	0	0	1.1	0	0	0	0	0	0.1	0	1	1
0.8	0.9	0.8	1.0	0.4	0.8	0	0.8	1.3	0	0	1	1
0.7	0.1	0	0.4	0	0	0	0	0	0.1	0	1	1
0.3	0.3	0	0.1	0.4	0	0	0	0	0.1	0.4	2	2
0	0.5	0	0	0.6	0.3	0	0	0	0.1	0.5	2	2
0	0	0	0	0	0	0	0	0	0	0	0	1
0	0.2	0	0	0.4	0.2	0	0.5	1.0	0.1	0,6	2	2
0	0	0	0	0.6	0.3	0	0	0.3	0.1	0.5	2	2
0.8	0.3	0	0.3	0	0	0	0.3	0	0	0	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0.1	0	0	0	0	0	0	0	0	0	0	0
0.2	0.4	0	0	0.4	0.2	0	0	0.5	0	0.3	2	2
0	0	0	0.1	0.2	0	0	0	0	0	0	?	1
0.9	0.4	0	0	0	0	0	0	0	0.2	0.1	1	1
0	0.2	0	0	0.1	0	0	0.5	0.6	0.1	0.3	2	2
0	0	0	0	0.5	0	0	0	0.2	0	0.1	2	2
0	0.2	0	0	0.6	0	0	0	0.1	0	0.1	2	2
0.1	0	0	0.3	0.1	0.3	0	0	1.0	0.9	0.1	1	1
0	0	0	0.4	0	0	0	0	0.5	0.2	0	1	1
0	0	0	0.5	0	0	0	0	0.1	0.1	0	1	1
0	0.2	0.1	0.2	0.2	0	0	0.2	0	0	0	?	0
0	0.4	0	0	0	0	0	0	0	0	0	?	1
0	0	0	0	0	0	0	0	0	0	0	0	0
0.3	0.6	0	0.3	0	0.1	0	0	0.2	0	0	?	1
0.1	0.5	0	0	0.1	0	0	0	0	0	0.1	2	2
0.4	0	0	0.3	0	0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0	0	0	0	0	0

Frequency of reactivity (absorbance-difference >0.3) with 38 Swedish blood donor sera.

1/38 0/38 0/38 0/38 0/38 0/38
0/38 0/38 0/38 0/38 0/38

Explanation: 0: control serum, 1: HTLV-I positive serum, 2: HTIV-II positive serum, ? serum with an uncertain reactivity.

Improved type discrimination with a combination of the results from an HTLV-I and an HTLV-II peptide.

As can be seen in table 1, EIA with a single peptide could not clearly differentiate between HTLV-I and HTLV--II. We then tried to analyze data in a two-dimensional diagram. At least two peptide pairs proved to give a relatively good type-specific discrimination (Fig 1 and 2). However, even with these pairs there were a few discrepancies.

Automatic interpretation of HTLV serotype:

To further improve the discrimination between the two HTLV-types we tried to take all results into account by multiplying the absorbances with weights according to the relative ability to discriminate for each peptide parameter. The weighted absrobances were then used for calculation of "HTLV-I-" and "HTLV-II-" points, respectively. The operations were performed in accordance with a computer program written in dBASE II as follows.

```
*HTLVPARS.CMD, A ROUTINE FOR SEROLOGICAL TYPING SERA
*INTO HTLV-I AND -II POSITIVITY.
SET INTENSITY OFF
SET TALK OFF
CLEAR
STORE "OK" TO MINDT
SET ALTERNATE TO HTLVTYP.TXT
DO WHILE MINDT="OK"
ERASE
É  1, 0 SAY "-------------------------------------------------"
É  1,50 SAY "-------------------------------"
É  2,24 SAY "PROGRAM FOR HTLV-TYPING OF SERA"
É  3, 0 SAY "-------------------------------------------------"
É  3,50 SAY "-------------------------------"
READ
STORE "        " TO MDBAS
É  5,5  SAY "Which is the name of the database? (S=stop) "GET MDBAS
READ
IF MDBAS="  "
 LOOP
ENDIF
IF !(MDBAS)="S"
 QUIT
ENDIF
STORE TRIM(!(MDBAS))+".DBF" TO MFDBAS
IF .NOT. FILE("&MDBAS")
 LOOP
ENDIF
CLEAR GETS
SET PRINT OFF
USE &MDBAS
SET ALTERNATE ON
SET CONSOLE OFF
? CHR(12)
? "       Results of the HTLV-typing of the serum samples registered in "
? " "
? "                              the database "+MDBAS+"."
? " "
? "Nr- Sample-----    Result-------------------------------------------"
? " "
SET CONSOLE ON
SET ALTERNATE OFF
DO WHILE .NOT. EOF
 É 7,5 SAY STR(#,3)
 É 9,5 SAY "Serum number "+AOMR+" "+YR+" "+STR(VAL(LABNO),5)+" being tested."
 É 11,5 SAY "
                                                                          "+;
  "               "
 READ
 STORE 0.0 TO HTLVPOINT
 STORE 0.0 TO HTLV1POINT
 STORE 0.0 TO HTLV2POINT
 IF G2:117>0.20
  IF (G1:111/G2:117)>=1.4
   STORE HTLV1POINT+1 TO HTLV1POINT
  ENDIF
```

```
 IF (G1:111/G2:117)<=0.6
  STORE HTLV2POINT+1 TO HTLV2POINT
 ENDIF
ENDIF
IF G1:111>0.3.AND.G2:117<0.1
 STORE HTLV1POINT+1 TO HTLV1POINT
ENDIF
IF G1:111>0.3.AND.G2:117>0.3
 STORE HTLVPOINT+0.5 TO HTLVPOINT
ENDIF
IF G2:398>0.3
 IF G1:392/G2:398<0.5
  STORE HTLV2POINT+0.5 TO HTLV2POINT
 ENDIF
ENDIF
IF E2:186>0.20
 IF E1:190/E2:186<0.6
  STORE HTLV2POINT+1 TO HTLV2POINT
 ENDIF
 IF E1:190/E2:186>=1.4
  STORE HTLV1POINT+1 TO HTLV1POINT
 ELSE
  IF E1:190/E2:186>2.5
  STORE HTLV1POINT+2 TO HTLV1POINT
  ENDIF
 ENDIF
ELSE
 IF E1:190>0.5
  STORE HTLV1POINT+1 TO HTLV1POINT
 ENDIF
ENDIF
IF E1:290>0.25
 STORE HTLVPOINT+1 TO HTLVPOINT
ENDIF
IF E1:380>0.25
 STORE HTLVPOINT+1 TO HTLVPOINT
ENDIF
IF D1:24>0.25
 IF (D1:19/D1:24)>1.9
  STORE HTLV1POINT+1 TO HTLV1POINT
 ENDIF
ENDIF
IF (D1:19+D1:24)>2
 STORE HTLVPOINT+1 TO HTLVPOINT
ENDIF
IF D1:19>5
 STORE HTLVPOINT+0.5 TO HTLVPOINT
ENDIF
REPLACE HT1 WITH HTLV1POINT, HT2 WITH HTLV2POINT,;
 HT  WITH HTLV1POINT+HTLV2POINT+HTLVPOINT
IF HT>1.0
 IF HT>3.5
  STORE "A clear" TO MEPITHET
 ELSE
  STORE "A" TO MEPITHET
```

```
 ENDIF
 DO CASE
  CASE HTLV1POINT>HTLV2POINT.AND.HTLV1POINT>1
   REPLACE TYPE WITH "1"
   STORE MEPITHET+" serological reactivity corresponding to HTLV-I.";
    TO MTYPECOM
  CASE HTLV1POINT>HTLV2POINT
   REPLACE TYPE WITH "1?"
   STORE MEPITHET+" serological reactivity resembling that of HTLV-I.";
    TO MTYPECOM
  CASE HTLV1POINT=HTLV2POINT
   REPLACE TYPE WITH "HT"
   STORE MEPITHET+" reactivity compatible with both HTLV-I and HTLV-II ";

    TO MTYPECOM
  CASE HTLV1POINT<HTLV2POINT.AND.HTLV2POINT>1
   REPLACE TYPE WITH "2"
   STORE MEPITHET+" serological reactivity corresponding to HTLV-II.";
    TO MTYPECOM
  CASE HTLV1POINT<HTLV2POINT
   REPLACE TYPE WITH "2?"
   STORE MEPITHET+" serological reactivity resembling that of HTLV-II.";

    TO MTYPECOM
 ENDCASE
ELSE
 REPLACE TYPE WITH "00"
 STORE "The serological reactivity was too weak for typing." TO MTYPECOM

ENDIF
É 11,5  SAY MTYPECOM
READ
SET ALTERNATE ON
SET CONSOLE OFF
? STR(#,3)+"  "+AOMR+" "+YR+" "+STR(VAL(LABNO),5)+"    "+MTYPECOM
SET CONSOLE ON
SET ALTERNATE OFF
SKIP
ENDDO
ENDDO
RETURN
```

The result is shown in figure 3.

In four cases the typing result was "not typable". Two of these sera were earlier classified as HTLV-antibody negative and two were earlier typed as weakly HTLV-I reactive. Thus, in no case the peptide-typing result was clearly different from the known or probable result. Judging from this a serotyping according to our technique would not lead to false typing results, but to a small number of results in the categories "not typable", or "HTLV of indeterminate type".

DISCUSSION OF THE RESULTS OF THE TEST SERIES.

Immunogenicity of HTLV proteins

The HTLV-I and -II genomes are 50% similar at the nucleic acid level (6, 10). The similarity is larger in gag than in env. Obvious similarities are however present also in env (10). Long type-specific sequences are present primarily in env. Within the two virus species the variation is very small. This means that peptides taken from one sequence potentially can detect antibodies in many infected persons provided that their sequence is immunogenic enough. The HTLV-antigens have both been studied with conventional serology (19, 17) and with monoclonal antibodies (8, 22, 27).

Serological reactivity in gag:

Palker et al (23) earlier showed that the C-terminus of HTLV-I p19 contains an important epitope, which reacts with certain monoclonal antibodies in a type-specific manner. The HTLV-I and -II peptide which we used in this work (1GB and 2GB) partially correspond to the peptide which Palker studied, but they are longer. We have in a larger serological material with our two peptides from this region found that antibodies against the C-terminus are very frequent in both HTLV-I and -II positive sera, and that the combinaton of our two peptides gives a better discrimination than each peptide in itself. Our longer peptides recreate the native conformation of p19 better

and has better possibilities to maintain it while bound to a solid phase, which is customary in many serological techniques. This is a prerequisite for performing the type discrimination analysis in a practical way.

We have found several other sequences in gag from HTLV-I which react with antibodies from both HTLV-I and -II seropositive persons (primarily 2GF, to a lesser extent 1GA and 2GA, data not shown). These function as general serological HTLV markers.

Serological reactivity in env:

We also found that the evolutionarily conserved sequences in gp21 (corresponding to peptides 1EC, 1ED and 1EE) could be used as type-common HTLV-serological markers. We found seven sera which reacted with a very conserved sequence (1ED), which is very similar to sequences in the murine leukemia virus p15E which probably has an immunosuppressive activity. This may have diagnostic implications and implications for the understanding of the pathogenesis of the diseases which are associated with HTLV (15).

It is known that the serological difference between HTLV-I and -II remains if a neutralization test with pseudotypes between VSV and HTLV is performed (10). This confirms that in the envelope there are important type--specific determinants (cf 19, 30). We have found one such determinant, here represented by the peptides 1EA and 2EA, which were derived from the outer envelope glycoprotein. In our series 10 of 15 HTLV-I positive sera and 8 of 10 HTLV-II positive sera reacted with their homologous counterpart of the two. It has been reported that human sera can react with a shorter HTLV-I peptide, which is contained within peptide 1EA, at a similar frequency (24). We found that as with the peptide pair 1GB and 2GB the combination of the peptides 1EA and 2EA was required for an optimal type discrimination. In 21 of 25 sera with known type the combination of the two peptides gave the right type. The four remaining sera reacted too weakly to

allow typing. No type-discordant reactivity was observed with this pair.

It is known from bovine leukosis virus (7) that the outer envelope glycoprotein (gp56) contains both linear and conformational epitopes. Some of them contribute to the neutralization of BLV. The antibodies which we demonstrate with the three gp56 peptides thus can also indirectly become useful for detection of neutralizing HTLV antibodies. Even the C-terminal peptide 1EB reacted relatively frequently (6 of 35 known HTLV positive sera). It was however not very type specific.

Our findings underline the type specificity of the outer glycoprotein, the most variable env-protein and of the C-terminus of p19m one of the most variable parts of gag. The STLV-I positive sera reacted mainly like the HTLV-I positive sera. The reactions with many of the env peptides were however relatively weak (cf 19, 29, 30). The high degree of similarity between these two viruses from different primate species, which then is reflected also at the peptide serological level (cf 12), indicates a common ancestry which is of more recent date than the common ancestry of HTLV-I and HTLV-II (29).

HTLV-I and -II as medical problems. The need for a stringent serological technique.

HTLV-I is a virus with an almost global distribution, even if the highest frequency of infected persons is present in southern Japan, the western Pacific, Carribean, Africa and southern Italy (6, 19). It is an important factor behind the diseases adult T-cell leukemia (6, 19) and tropical spastic paraparesis (21, 25). HTLV-II so far is associated with a few cases of hairy cell leukemia (6, 16, 20).

Gradually both HTLV-I and -II have become great medical problems also in countries with a relatively low percentage of infected persons. Both can be transmitted with blood, and in the USA and Japan HTLV-I antibodies are analyzed routinely in blood donations (32). Thus a large

need for confirmation of the serological screening results with as dependable methods as possible has been created. It has also become important to differentiate between HTLV-I and HTLV-II infection. The importance for the patient of differentiating between the two infections is however still uncertain. Both are associated with serious diseases. It is reasonable to assume that there are important differences in the degree and type of disease which may occur in the HTLV-I and HTLV-II positive patient.

In the USA recently a surprisingly high degree of HTLV-seropositivity was found in intravenous drug abusers (14, 26). When these sera were typed most of these reactions proved to be due to HTLV-II. HTLV-II earlier was considered very rare. It is unclear from where the virus has come. Also in great Britain (28) and Italy (11) HTLV of both types has been shown to occur in intravenous drug abusers.

Current technique for demonstration and typing of HTLV infection.

In spite of widespread use HTLV serology still is an incomplete tool for demonstration and typing of HTLV infection. A large part of the initially positive findings become negative at a comprehensive analysis. Weak and indeterminate reactivities are common. Therefore there are probably a not insignificant portion of false-negative results in the serology (3). However, a number of possibilities for confirmation of initally positive findings exist.

The techniques which now are available for typing of an HTLV infection comprise virus isolation with typing, western blot with HTLV-I and HTLV-II antigen, radioimmunoprecipitation assay (RIPA) with polyacrylamide gel electrophoresis and antigen from both viruses, neutralization assay with pseudotypes of both viruses and nucleic acid amplification, possibly followed by restriction enzyme analysis, hybridization or sequencing. In

western blot with HTLV-I antigen there are often few cross-reactions with HTLV-II on p19. In RIPA type specific reactions can be studied especially well. In competition RIPA type specific reactions have been demonstrated also on p24. PCR (polymerase chain reaction, a type of nucleic acid amplification) has proven to be of great potential for discriminating between the two viruses, but has so far required lymphocytes from the patient. These techniques all require comparatively much time and competence. A simple, cheap and rapid test is needed.

Computer-aided interpretation of multiparametric serological results.

The pattern of serological reactivity with synthetic peptides often is individual (15). Therefore the sensitivity is increased when results from several synthetic peptides are combined. In a commercial test one can sometimes mix the peptides directly in the analytical well, but this means that the qualitative contribution given by each peptide is ignored. By analyzing the reactivity of each peptide the sensitivity can be kept high without loss of specificity information. The above given computer program illustrates the principle. We have later modified the program somewhat and thereby acheived a somewhat better type discrimination. The program judges if a typing can be performed with the available information. If that is not the case this is indicated. If the number of HTLV-I and HTLV-II, respectively do not differ clearly the result is classified as "HTLV antibodies demonstrated. Typing not possible". If the number of points for a certain type is at least twice as high as the number of points for the other, that type is reported. The program can easily be modified. New peptides can easily be added when their general HTLV reactivity and ability to type discriminate become approximately known. The weighting factors may have to be modified continuously depending on the reactivity of controls and increasing experience. This pattern recognition problem can be treated in many ways, among others

with a learning machine approach, the multivariate analysis method and by the use of dichotomous parsing. However, these principles are not discussed here in detail. For practical reasons we have chosen a program which primarily works according to the third principle.

The new technique

The use of a panel of synthetic peptides gives a detailed insight into the immune response to HTLV, and complements other techniques for confirmation and typing of HTLV infection. Peptides from the envelope glycoprotein gene yielded a particularly good result. The reactivity with the envelope glycoproteins is often weak in western blot, but often strong in our peptide tests. The peptide tests thus give an opportunity to demonstrate antibody activity against both envelope (env) as well as internal (gag) components, which is an important criterion of true HTLV antibody activity.

Conclusion:

In 32 sera of 36 with known or probable HTLV type we were able to correctly decide whether a serum was HTLV-I or HTLV-II positive. The discrepant sera all gave very weak reactions.

Four additional peptides

In addition to the above synthesized and tested peptides, we synthesized, by a similar technique, the following four peptides:

a) HTLV-I gag 130-197 PVMHPHGAPPNHRPWQMKDLQAIKQEVSQAAPGSPQFMQTIRLAVQQFDPTAKDLQDLLQYLCSSLVA
b) HTLV-II gag 137-214 PILHPPGAPSAHRPWQMKDLQAIKQEVSSSALGSPQFMQTLRLAVQQFDPTAKDLQDLLQYLCSSLVV

a) HTLV-I gag 298-349 LRSLAYSNANKECQKLLQARGHTNSPLGDMLRACQTWTPKDKTKVLVVQPKK
b) HTLV-II gag 305-356 LRSLAYSNANKECQKILQARGHTNSPLGEMLRTCQAWTPKDKTKVLVVQPRR

Preliminary results support that these peptides, which are derived from p24 of HTLV-I and -II, can detect HTLV-I and HTLV-II antibodies and that they react in a type-specific way in an immunoassay according to the

present invention. The distinguishing feature of these peptides in that due to their length they simulate HTLV-specific epitopes better than shorter peptides.

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CLAIMS

1. A method of discriminating between specific antibodies in samples of sera or other body fluids from humans or other primates containing antibodies arising from infection with HTLV-I, containing antibodies arising from infection with HTLV-II or containing antibodies arising from infection with related retroviruses, c h a r a c t e r i z e d in that the sample to be analyzed is subjected to at least four immunoassays, each using a different diagnostic antigen selected from the following groups a) to d):

a) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I gag comprising antigenic structures;

b) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II gag comprising antigenic structures;

c) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I env comprising antigenic structures;

d) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II env comprising antigenic structures;

with the proviso that at least one peptide from each of the groups a) to d) is selected and, further, that at least one pair of peptides corresponding to at least partially overlapping sequences of HTLV-I and HTLV-II is selected from each of the groupages a) plus b), and c) plus d),

and that the analyzed, different binding strengths of the antibodies of the sample in said at least four immunoassays are used to discriminate between antibodies arising from infection with one specific retrovirus and antibodies arising from infection with other specific retroviruses.

2. A method according to claim 1, wherein the diagnostic antigens are selected from the peptides

a) HTLV-I gag 130-197 PVMHPHGAPPNHRPWQMKDLQAIKQEVSQAAPGSPQFMQTIRLAVQQFDPTAKDLQDLLQYLCSSLVA
b) HTLV-II gag 137-214 PILHPPGAPSAHRPWQMKDLQAIKQEVSSSALGSPQFMQTLRLAVQQFDPTAKDLQDLLQYLCSSLVV

a) HTLV-I gag 298-349 LRSLAYSNANKECQKLLQARGHTNSPLGDMLRACQTWTPKDKTKVLVVQPKK
b) HTLV-II gag 305-356 LRSLAYSNANKECQKILQARGHTNSPLGEMLRTCQAWTPKDKTKVLVVQPRR

a) HTLV-I gag 4-20 IFSRSASPIPRPPRGLA
b) HTLV-II gag 4-20 IHGLSPTPIPKAPRGLS

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

a) HTLV-I gag 265-285 SILQGLEEPYHAFVERLNIAL

a) HTLV-I gag 302-320 LAYSNANKECQKLLQARGH

a) HTLV-I gag 323-341 SPLGDMLRACQTWTPKDKT

a) HTLV-I gag 337-355 PKDKTKVLVVQPKKPPPNQ
b) HTLV-II gag 343-361 PKDKTKVLVVQPRRPPPTQ

a) HTLV-I gag 378-399 PCPLCQDPTHWKRDCPRLKPT

a) HTLV-I gag 392-411 DCPRLKPTIPEPEPEEDALL
b) HTLV-II gag 398-416 DCPQLKPPQEEGEPLLLDL

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

c) HTLV-I env 290-312 HNSLILPPFSLSPVPTLGSRSRR

c) HTLV-I env 360-378 AIVKNHKNLLKIAQYAAQN

c) HTLV-I env 376-392 AQNRRGLDLLFWEQGGL

c) HTLV-I env 380-398 RGLDLLFWEQGGLCKALQE

c) HTLV-I env 465-488 RQLRHLPSRVRYPHYSLILPESSL
d) HTLV-II env 463-486 IQALPQRLQNRHNQYSLINPETML

3. A method according to claim 2, wherein at least the following peptides are selected:

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

4. A method according to any one of claims 1-3, wherein the sample to be analyzed is subjected to at least eight immunoassays and the analyzed pattern of binding strengths is processed with a computer program.

5. A method according to any one of claims 1-4, wherein at least one of the selected peptides is attached to an inert soluble or insoluble carrier.

6. A peptide, c h a r a c t e r i z e d in that it corresponds to a sequence of HTLV-I, HTLV-II or a related retrovirus each comprising antigenic structures and that it comprises a sequence of at least 17 amino acid residues selected from the following sequences:

HTLV-I gag 130-197 PVMHPHGAPPNHRPWQMKDLQAIKQEVSQAAPGSPQFMQTIRLAVQQFDPTAKDLQDLLQYLCSSLVA
HTLV-II gag 137-214 PILHPPGAPSAHRPWQMKDLQAIKQEVSSSALGSPQFMQTLRLAVQQFDPTAKDLQDLLQYLCSSLVV

HTLV-I gag 298-349 LRSLAYSNANKECQKLLQARGHTNSPLGDMLRACQTWTPKDKTKVLVVQPKK
HTLV-II gag 305-356 LRSLAYSNANKECQKILQARGHTNSPLGEMLRTCQAWTPKDKTKVLVVQPRR

HTLV-I gag 4-20 IFSRSASPIPRPPRGLA
HTLV-II gag 4-20 IHGLSPTPIPKAPRGLS

HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

HTLV-I gag 265-285 SILQGLEEPYHAFVERLNIAL

HTLV-I gag 302-320 LAYSNANKECQKLLQARGH

HTLV-I gag 323-341 SPLGDMLRACQTWTPKDKT

HTLV-I gag 337-355 PKDKTKVLVVQPKKPPPNQ
HTLV-II gag 343-361 PKDKTKVLVVQPRRPPPTQ

HTLV-I gag 378-399 PCPLCQDPTHWKRDCPRLKPT

HTLV-I gag 392-411 DCPRLKPTIPEPEPEEDALL
HTLV-II gag 398-416 DCPQLKPPQEEGEPLLLDL

HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

HTLV-I env 290-312 HNSLILPPFSLSPVPTLGSRSRR

HTLV-I env 360-378 AIVKNHKNLLKIAQYAAQN

HTLV-I env 376-392 AQNRRGLDLLFWEQGGL

HTLV-I env 380-398 RGLDLLFWEQGGLCKALQE

HTLV-I env 465-488 RQLRHLPSRVRYPHYSLILPESSL
HTLV-II env 463-486 IQALPQRLQNRHNQYSLINPETML

7. A method of detecting antibodies arising from infection with HTLV-I, HTLV-II or a related retrovirus in a sample of serum or other body fluid from a human or an other primate, c h a r a c t e r i z e d in that said sample is subjected to an immunoassay using as a diagnostic antigen at least one peptide according to claim 6.

8. An immunoassay kit for the discrimination between specific antibodies in samples of sera or other body fluids from humans or other primates containing antibodies arising from infection with HTLV-I, containing antibodies arising from infection with HTLV-II or containing antibodies arising from infection with related retroviruses, c h a r a c t e r i z e d in that it comprises at least four peptides selected from the following groups a) to d):

a) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I gag comprising antigenic structures;

b) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II gag comprising antigenic structures;

c) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-I env comprising antigenic structures;

d) peptides comprising a sequence of at least 17 amino acid residues which corresponds to a sequence of HTLV-II env comprising antigenic structures;

with the proviso that it comprises at least one peptide from each of the groups a) to d) and, further, that it comprises at least one pair of peptides corresponding to at least partially overlapping sequences of HTLV-I and HTLV-II from each of the groupages a) plus b), and c) plus d).

9. An immunoassay kit according to claim 8, wherein it comprises at least four peptides selected from the peptides

a) HTLV-I gag 130-197 PVMHPHGAPPNHRPWQMKDLQAIKQEVSQAAPGSPQFMQTIRLAVQQFDPTAKDLQDLLQYLCSSLVA
b) HTLV-II gag 137-214 PILHPPGAPSAHRPWQMKDLQAIKQEVSSSALGSPQFMQTLRLAVQQFDPTAKDLQDLLQYLCSSLVV

a) HTLV-I gag 298-349 LRSLAYSNANKECQKLLQARGHTNSPLGDMLRACQTWTPKDKTKVLVVQPKK
b) HTLV-II gag 305-356 LRSLAYSNANKECQKILQARGHTNSPLGEMLRTCQAWTPKDKTKVLVVQPRR

a) HTLV-I gag 4-20 IFSRSASPIPRPPRGLA
b) HTLV-II gag 4-20 IHGLSPTPIPKAPRGLS

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

a) HTLV-I gag 265-285 SILQGLEEPYHAFVERLNIAL

a) HTLV-I gag 302-320 LAYSNANKECQKLLQARGH

a) HTLV-I gag 323-341 SPLGDMLRACQTWTPKDKT

a) HTLV-I gag 337-355 PKDKTKVLVVQPKKPPPNQ
b) HTLV-II gag 343-361 PKDKTKVLVVQPRRPPPTQ

a) HTLV-I gag 378-399 PCPLCQDPTHWKRDCPRLKPT

a) HTLV-I gag 392-411 DCPRLKPTIPEPEPEEDALL
b) HTLV-II gag 398-416 DCPQLKPPQEEGEPLLLDL

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

c) HTLV-I env 290-312 HNSLILPPFSLSPVPTLGSRSRR

c) HTLV-I env 360-378 AIVKNHKNLLKIAQYAAQN

c) HTLV-I env 376-392 AQNRRGLDLLFWEQGGL

c) HTLV-I env 380-398 RGLDLLFWEQGGLCKALQE

c) HTLV-I env 465-488 RQLRHLPSRVRYPHYSLILPESSL
d) HTLV-II env 463-486 IQALPQRLQNRHNQYSLINPETML

10. An immunoassay kit according to claim 9, wherein it comprises at least the following peptides:

a) HTLV-I gag 111-130 PDSDPQIPPPYVEPTAPQVL
b) HTLV-II gag 117-136 PSPEAHVPPPYVEPTTTQCP

c) HTLV-I env 190-213 LLPHSNLDHILEPSIPWKSKLLTL
d) HTLV-II env 186-209 LVHDSDLEHVLTPSTSWTTKILKF

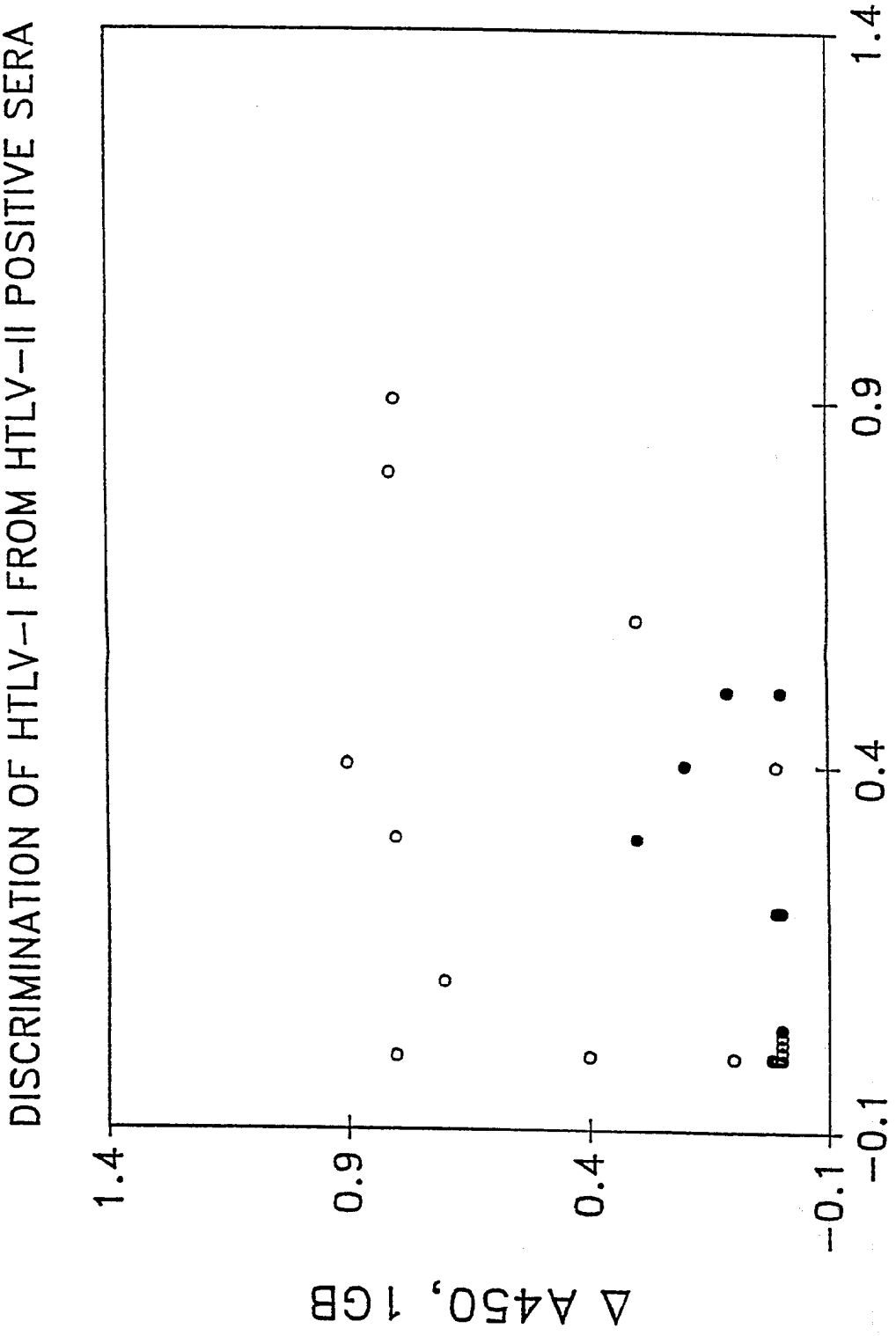
DISCRIMINATION OF HTLV-I FROM HTLV-II POSITIVE SERA
Δ A450, 1GB
Δ A450, 2GB
1.4
0.9
0.4
-0.1

FIG.1

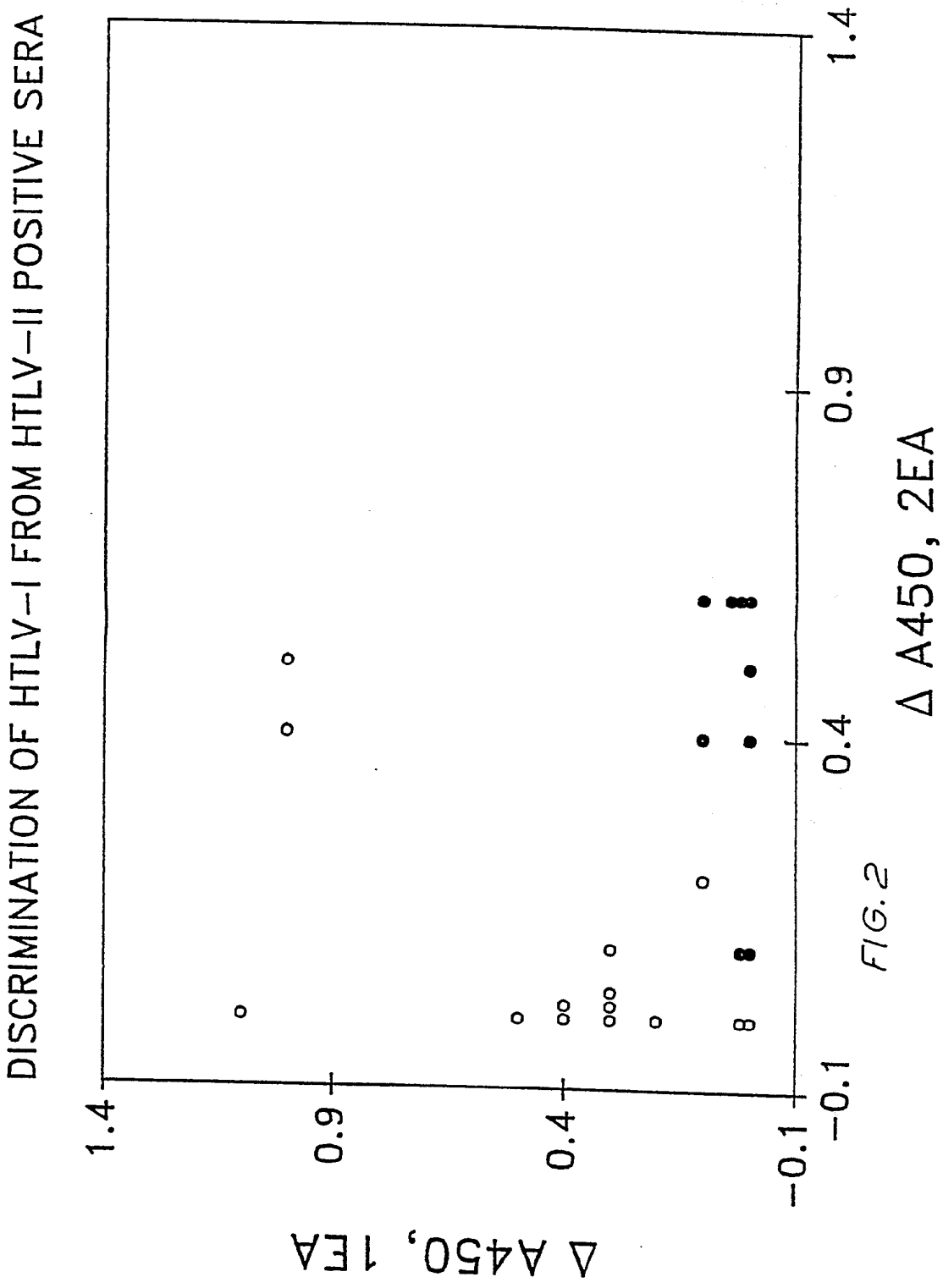
DISCRIMINATION OF HTLV-I FROM HTLV-II POSITIVE SERA
Δ A450, 1EA
Δ A450, 2EA
1.4
0.9
0.4
-0.1

FIG. 2

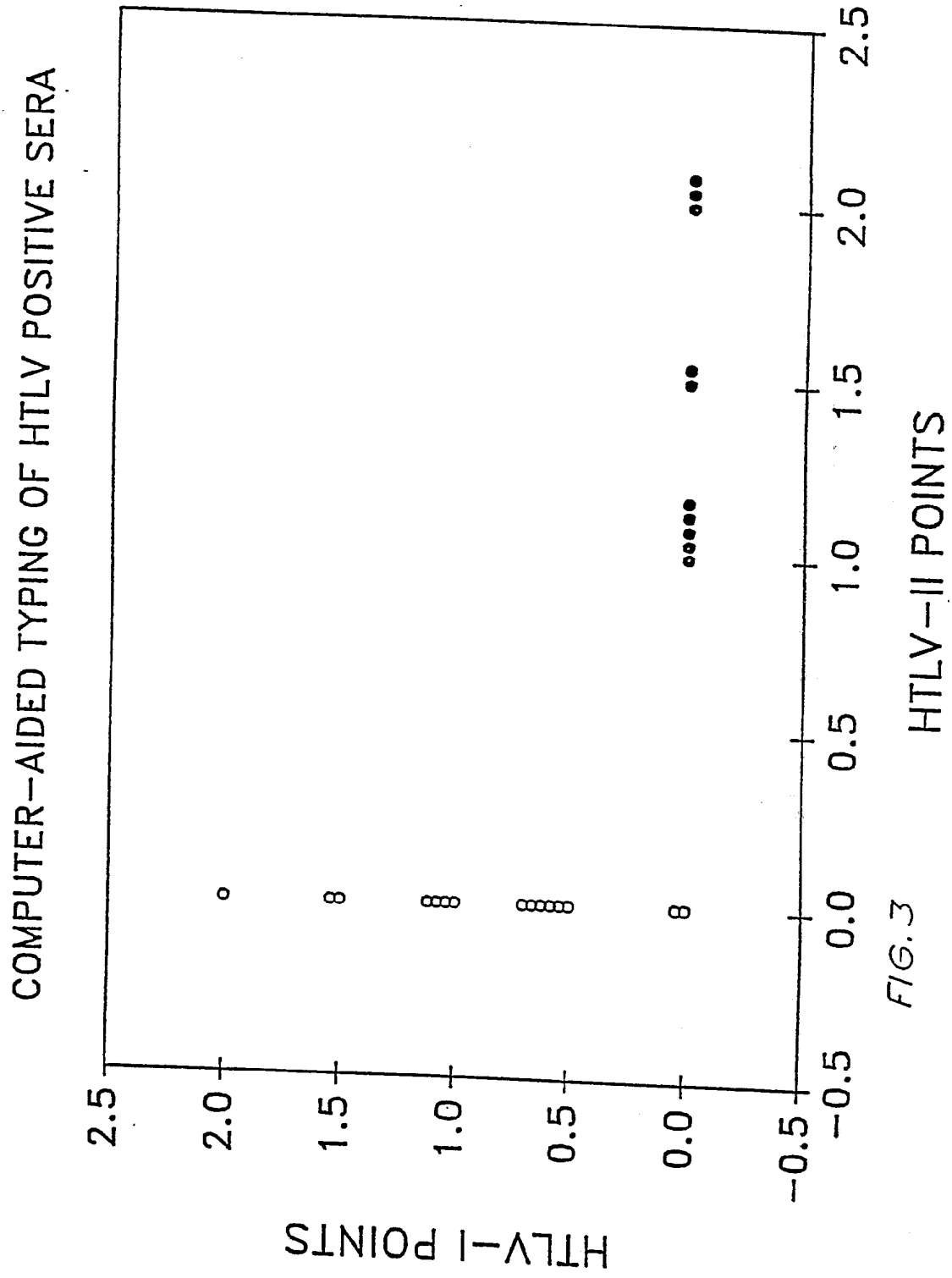
COMPUTER-AIDED TYPING OF HTLV POSITIVE SERA
HTLV-I POINTS
HTLV-II POINTS
2.5
2.0
1.5
1.0
0.5
0.0
-0.5

FIG. 3

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 90/00139

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 33/569, 33/543, C 07 K 7/10

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y	WO, A1, 89/01527 (CELLULAR PRODUCTS, INC.) 23 February 1989, see the whole document --	1-5,7-10
Y	WO, A1, 86/01834 (THE REGENTS OF THE UNIVERSITY OFCALIFORNIA) 27 March 1986, see in particular pages 12-22 and claims 37-9 --	1-5,7-10
A	The Journal of Immunology, Vol. 135, No. 1, July 1985 T J Palker et al.: "Monoclonal antibodies reactive with human t cell lymphotropic virusl (htlv1) p19 internal core protein: cross-reactivitywith normal tissues and differential reactivity with htlv types I and II1. ", see page 247 --	1-10

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1st June 1990	1990 -06- 11
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Carl-Olof Gustafsson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Nature, Vol. 329, September 1987 E Norrby et al.: "Discrimination between antibodies to HIV and to related retroviruses using site-directed serology. ", see page 248 --	1-10
A	EP, A2, 0267622 (KYOWA HAKKO KOGYO CO., LTD.) 18 May 1988, see the whole document --	1
X	The Journal of Immunology, Vol. 142, No. 3, February 1989 T J Palker et al.: "Mapping of immunogenic regions of human t cell leukemia virus type I (HTLV-I) gp46 and gp21 envelope glycoproteins with env-encoded synthetic peptides and a monoclonal antibody to gp461. ", see page 971 - page 978 and table I peptides 5-7 and 10-11	6
Y	--	1-5,7-10
Y	US, A, 4689398 (YING-JYE WU ET AL.) 25 August 1987, see claim 2	1-5,7-10
X	--	6
X	US, A, 4525300 (M YOSHIDA ET AL.) 25 June 1985, see claims	6
Y	--	1-5,7-10
X	US, A, 4804746 (M YOSHIDA ET AL.) 14 February 1989, see claims	6
Y	--	1-5,7-10
P,X	WO, A1, 89/08664 (VIROVAHL S.A.) 21 September 1989, see claim and page 11, lines 5-18 --	6

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 90/00139

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 90-05-07
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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US-A- 4689398	87-08-25	JP-A-	61030600	86-02-12
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		EP-A-B-	0107053	84-05-02
		JP-A-	59128366	84-07-24
		US-A-	4804746	89-02-14
		JP-A-	59155347	84-09-04
US-A- 4804746	89-02-14	CA-A-	1262014	89-09-26
		EP-A-B-	0107053	84-05-02
		JP-A-	59128366	84-07-24
		US-A-	4525300	85-06-25
		JP-A-	59155347	84-09-04
WO-A1- 89/08664	89-09-21	NONE		